

upon temperature and upon concentration, decreasing as the temperature is raised from 2°C to 25°C, and as the solution is diluted, as one expects for an equilibrium of the type  $A + B \rightleftharpoons C$ .

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<sup>13</sup> Barondes, S., and M. Nirenberg, *Science*, **138**, 813 (1962).

<sup>14</sup> Spyrides, G., and F. Lipmann, these PROCEEDINGS, **48**, 1977 (1962).

<sup>15</sup> Gilbert, W., *J. Mol. Biol.*, in press.

<sup>16</sup> Ishihama, A., N. Mizuno, M. Takai, E. Otaka, and S. Osawa, *J. Mol. Biol.*, **5**, 251 (1962).

<sup>17</sup> Risebrough, R., A. Tissières, and J. Watson, these PROCEEDINGS, **48**, 430 (1962).

<sup>18</sup> Warner, J., A. Rich, and C. E. Hall, *Science*, **138**, 1339 (1962).

<sup>19</sup> Warner, J., P. Knopf, and A. Rich, these PROCEEDINGS, **49**, 122 (1963).

<sup>20</sup> Cannon, M., R. Krug, and W. Gilbert, unpublished results.

## THE MAINTENANCE OF THE ACCURACY OF PROTEIN SYNTHESIS AND ITS RELEVANCE TO AGEING

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The ways in which the accumulation of mutations might contribute to the process of ageing in higher organisms or in individual clones of cells has been discussed at length.<sup>1</sup> No corresponding treatment of the consequences of transcription errors in the translation of the DNA message into RNA and protein sequences seems to be available. Here I show that a consideration of the rate of accumulation of such errors leads to a paradox, the resolution of which may be relevant to the problem of ageing, and that there are a number of simple experiments which should decide whether this is the case.

The basic idea is a simple one, namely, that the ability of a cell to produce its complement of functional proteins depends not only on the correct genetic specification of the various polypeptide sequences, but also on the competence of the protein-synthetic apparatus. A cell inherits, in addition to its genetic DNA, the enzymes necessary for the transcription of that material into polypeptide sequences; the inheritance of inadequate protein-synthesizing enzymes can be as disastrous as the inheritance of a mutated gene. Similarly, a cell may deteriorate through a progressive decrease in the adequacy of its transcription mechanism, just as it may through the accumulation of somatic mutations.

As a basis for further discussion we shall suppose:<sup>2</sup> (a) that the sequence of amino acids in a protein is determined by the sequence of nucleotides in a corresponding region of the DNA; (b) that the immediate functions of DNA include the direction of the synthesis of transfer, microsomal, and messenger RNA but not of proteins; and (c) that the information for peptide sequence determination is carried by messenger RNA, but that other forms of RNA may affect the accuracy of protein synthesis.

There is little direct evidence concerning the accuracy of polypeptide-sequence determination. The accuracy of synthesis of messenger RNA is unlikely to exceed that of DNA replication, for which an error frequency of  $10^{-8}$  per base has been estimated.<sup>1</sup> This would contribute an error frequency of about  $3 \times 10^{-8}$

to protein synthesis if a degenerate three-letter code is assumed. However, the process of sequence determination also involves the specific reaction of the amino acids with their activating enzymes; this is likely to be a more important source of error. It must be difficult to distinguish a pair of amino acids as similar as valine and isoleucine even with an error level of  $10^{-4}$ .

Our lack of knowledge of the error-frequency in protein synthesis makes it impossible to say, *a priori*, whether or not the accumulation of errors of protein sequence is relevant to the processes of ageing in higher organisms; this point must be decided experimentally. The nature of the mechanisms by means of which the accuracy of protein synthesis is maintained remains of interest, however low the error frequency. The first part of this paper will be concerned with these mechanisms.

We consider the following problem: suppose we could obtain a cell in which initially every polypeptide chain was in exact correspondence with its DNA sequence, the correspondence being assumed unambiguous. What would be the consequences of errors in protein transcription? We shall concentrate on what must be one of the main types of transcription error, namely, the replacement of a given amino acid by an incorrect amino acid. Let us roughly (and necessarily somewhat arbitrarily) divide the proteins of the cell into two sets, the first concerned with physical structure and intermediary metabolism, and the other concerned with the processing of genetic information, that is, the determination of the sequences of nucleic acids and polypeptides. The consequences of transcription errors for the two classes are very different.

If a small proportion of the protein molecules responsible for some metabolic function, say a part of glucose metabolism, were in error, then we might expect a somewhat lower *average* turnover number for the enzyme, a slightly reduced *average* specificity of the reaction, or perhaps a slight loosening of the *average* "control" of the reaction by feed-back inhibition, etc. These effects would not be cumulative; once the faulty messenger RNA or protein had been degraded, all memory of the error would soon be lost.

The situation is very different for the second class of proteins. Errors leading to complete loss of function would again affect only the efficiency of the information-transfer process in terms of average number of polymers replicated or synthesized per enzyme molecule. However, errors which lead to a reduced specificity of an information-handling enzyme lead to an increasing error frequency. Such processes are clearly cumulative and arguments which we shall next give in some detail suggest that, in the absence of an imposed selection for "accurate" protein-synthesizing units, must lead ultimately to an error catastrophe; that is, the error frequency must reach a value at which one of the processes necessary for the existence of viable cell becomes critically inefficient.

Under any given conditions the initial error frequency corresponding to the replacement of a particular amino acid in a particular position in a protein must be well-defined. Naturally it depends both on the amino acid replaced, the new and incorrect amino acid substituted (or, more correctly, on their base representations in the nucleic acid), and perhaps also on the environment in the peptide. Some replacements must certainly be more frequent than others on account of specificity at the transfer-enzyme level; further variety is introduced by any nonran-

dom distribution of errors of RNA synthesis. Similarly, each error in the protein-synthesizing system will induce a quite specific family of errors in the material synthesized; a loss of amino acid specificity of the phenylalanine activating enzyme, for example, could only effect substitutions involving the replacement of phenylalanine.

One feature of the dependence of the frequency of induced errors on the concentration of errors already present seems fairly certain, namely, that for small enough concentrations of errors the principal term is linear; that is, if we double the number of each kind of error already present in the protein-synthesizing system, we shall double the excess of induced errors over that in the initial error-free system. We shall not lose sight of the general features of the problem if, instead of considering the development of errors class by class, we lump them together and consider the error frequency  $p$  measured as the proportion of errors per amino acid present in polypeptide.

New protein synthesized by the initially correct enzymes will have some characteristic error concentration, say  $p_0$ . In the absence of any influence of errors already present on the frequency of error production, the error-level would settle down to  $p_0$  once an amount of protein, large compared with that initially present, had been synthesized. If a slight linear dependence of the frequency of error production on the concentration of errors already present must be taken into account, we may, as a first approximation, write

$$\frac{dp}{dt} = \alpha p \quad (1)$$

where we consider the development of the system only after a time  $t_0$  at which the error frequency  $p_0$  has become established. This equation is only valid if  $p$  is small.

The solution of equation (1),  $p = p_0 e^{\alpha t}$ , shows that the error frequency initially increases exponentially and hence we predict the error catastrophe already mentioned. A proper treatment of this problem would take account of the time delay in the expression of errors. More importantly, the methods of probability theory should be used to determine the variance of  $p$ , etc.; the use of the differential equation (1) may be a rather poor approximation here, since the number of errors per cell may be small. These elaborations do not seem justified in the absence of more detailed experimental evidence.

How could an error catastrophe be avoided? Genetic selection for these proteins of DNA sequences, which in the course of transcription give particularly low error frequencies, may be possible within limits; selection for sequences which are completely inactivated by amino acid substitution would be more powerful. Selective scavenging of all incorrect proteins by hydrolytic enzymes might also contribute. However, in the light of recent work on ambiguous mutants,<sup>3</sup> it seems unlikely that these methods are powerful enough. What is needed is a selection based on the accuracy of protein synthesis, that is, a selection which rejects enzymes which lead to too many errors in protein synthesis. This could be achieved within a single cell only by a partial or complete segregation of the products of one piece of "protein-synthetic apparatus." All the available evidence argues against such segre-

gation. We must presume then that the selection works at a cellular or higher level.

In any population of cells there must be a certain variance in the accuracy of the protein-synthetic apparatus so that a sufficiently severe selective pressure could eliminate the least accurate cells; otherwise, all cell lines would ultimately deteriorate. I shall try to put this result in a paradoxical form as follows: suppose we take a single bacterial cell and culture it under "ideal" conditions. After the first division we choose one daughter cell at random, allow it to divide again, choose one daughter cell at random, and proceed in this way in the absence of any "competition" between cells.<sup>4</sup> Then the arguments given above show that we finally get a nondividing cell even in the absence of mutation (naturally, the mutation frequency would also increase when the error frequency rose sufficiently). If these arguments are correct, one of the important functions of selection at cellular level is the maintenance of the accuracy of protein synthesis. Such a function would be additional to that usually discussed by geneticists and might quite possibly be equally important.

These speculations raise two important questions when applied to higher organisms. Firstly, does a cumulative inaccuracy in protein synthesis, connected with mutation only in so far as it is likely to lead to an increased somatic-mutation rate, play any role at all in the clearly extremely complex phenomenon of ageing? Secondly, what are the protective and selective mechanisms which prevent the error catastrophe in higher organisms?

There seems a fairly obvious experimental approach to the first of these questions. If we wish to study the effect of errors in protein synthesis in the absence of complications due to *primary* nucleic acid changes, we must increase such errors specifically, that is, without affecting nucleic acid synthesis. This could now be done by incorporating appropriate amino acid analogues. In the case of microorganisms of fairly representative selection of amino acid, errors could be induced by adding subtoxic concentrations of, say, *p* fluorophenylalanine, or, better, a mixture of *p* fluorophenylalanine and ethionine,<sup>5</sup> to the growth medium. A related experiment could almost certainly be done with mice. The level of incorporation of the analogue in these experiments should be far below that which causes death directly by gross inactivation of enzymes. In principle a pulse of analogue should produce consequences which cannot be reversed (except by selection) even after elimination of the analogue. It is perhaps worth remarking that it should be possible to study the effects of point mutations and primary errors of RNA synthesis by using bromouracil and fluorouracil, respectively, instead of amino acid analogues.

In higher organisms the situation is complicated by the existence of dividing and nondividing cells. The accumulation of transcription errors is likely to be particularly serious for the latter since, in the absence of division, selection at the cellular level is impossible. In slowly dividing tissue, selection may or may not be able to maintain the accuracy of protein synthesis; this can only be determined by experiment.

Now we come to the mechanism of protection or selection in higher organisms. If any part of the ageing process has to do with the accumulation of errors of polypeptide sequence, we need to know how it is that each new organism comes to have as clear a start as its parents. Separation of the germ line early may help in

some way, but we have no reason to believe that the *enzymes* initially present in the egg have been subjected to a significantly reduced possibility of error. Various solutions seem possible; for example, selection in the growing embryo may be strong enough or the process of embryogenesis may demand such a high accuracy that its successful completion guarantees the necessary accuracy of protein synthesis. One can also conceive of special mechanisms of quality control; for example, special proteins might be synthesized which are converted by a certain class of errors into lethal polypeptides. This would guarantee that the frequency of this class of errors in viable cells is kept low. At present there is no evidence available which enables one to select among these possibilities.

I wish to make it quite clear that I am not proposing here that the accumulation of protein transcription errors is "the mechanism of ageing." My object is the more modest one of pointing out one source of progressive deterioration of cells and cell lines. Since I am unable to estimate the time scale of this process, I can only suggest experiments which should show where, if anywhere, it contributes to the ageing process in higher organisms.

I am indebted to Professor H. C. Longuet-Higgins and Dr. F. H. C. Crick for valuable criticisms of my original manuscript.

<sup>1</sup> Szilard, L., these PROCEEDINGS, 45, 30 (1959); Maynard Smith, J., *Proc. Roy. Soc.*, B157, 115 (1962).

<sup>2</sup> Perutz, M., *Proteins and Nucleic Acids: Structure and Function* (Amsterdam: Elsevier, 1962).

<sup>3</sup> Benzer, S., and S. P. Champe, these PROCEEDINGS, 47, 1025 (1961); 48, 532 (1962).

<sup>4</sup> Note that in an exponentially growing culture there is a strong selection for cells with a short division time.

<sup>5</sup> See Kempner, E. S., and D. B. Cowie, *Biochim. et Biophys. Acta*, 42, 401 (1960) and references therein.

## DYNAMIC ROLE OF TRIPLET STATES IN PHOTOSYNTHESIS\*

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The purpose of this communication is to point out some attractive features of primary photosynthetic steps based, partially at least, on *energy transfer* and *energy multiplication* mechanisms which involve the triplet state of chlorophyll. A more critical comparison of triplet state mechanisms, singlet state mechanisms, and photoionization mechanisms will be deferred until a later time. Here we strive only to correct some misunderstandings concerning the possible role of triplet states in photosynthesis and to speculate upon certain mechanisms often excluded from past thinking because of the misunderstandings.

*Population of Triplets.*—There seems little doubt that efficient singlet  $\rightarrow$  triplet radiationless transitions (intersystem crossing) can occur in molecular aggregates, providing processes such as radiation or chemical quenching which limit the lifetime of the singlet state are not too fast. In the case where excitation transfer is